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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
RAMIREZ, DELIA M	

ART UNIT	PAPER NUMBER
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/656,531	BALTIMORE ET AL.	
	Examiner	Art Unit	
	DELIA RAMIREZ	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 December 2010*.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *21,28,43,109-113,120-135 and 137-143* is/are pending in the application.

4a) Of the above claim(s) *43,109-113,120-135 and 137-143* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *21 and 28* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *12/1/10*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status of the Application

Claims 21, 28, 43, 109-113, 120-135 and 137-143 are pending.

A request for continued examination under 37 CFR 1.114 was filed in this application after a decision by the Board of Patent Appeals and Interferences, but before the filing of a Notice of Appeal to the Court of Appeals for the Federal Circuit or the commencement of a civil action. Since this application is eligible for continued examination under 37 CFR 1.114 and the fee set forth in 37 CFR 1.17(e) has been timely paid, the appeal has been withdrawn pursuant to 37 CFR 1.114 and prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on 12/1/2010 is acknowledged.

Applicant is advised that claim 40 has been cancelled per applicant's own request as shown in the appeal brief filed on 3/2/2009. See part IV, Status of the Amendment, where applicant specifically requested cancellation of claim 40. As indicated in the Examiner's answer of 7/7/2009, that amendment was entered. See part (3) of the answer. Therefore, claim 40 is not part of the list of pending claims and will not be examined. Claims 43, 109-113, 120-135 and 137-143 are withdrawn from consideration as being directed to non-elected subject matter. Claims 21 and 28 are at issue and are being examined herein.

Information Disclosure Statement

1. The information disclosure statement (IDS) submitted on 12/1/2010 is acknowledged. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Objections

2. The claims are objected to due to the recitation of "binds to a target site in an endogenous mammalian gene…….region flanking a target sequence in chromosomal DNA". While one would understand that the endogenous mammalian gene is the chromosomal DNA being referred to, for consistency and to avoid confusion, it is suggested that the term be amended to recite, for example, "region flanking a target site in the endogenous mammalian gene". Appropriate correction is required.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claims 21, 28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Choulika et al. (U.S. Publication No. 20020107214, U.S. Application No. 10/917295 filed on 7/27/2001) in view of Bibikova et al. (Molecular and Cellular Biology 21(1):289-297, 2001) and further in view of Takeuchi et al. (Biochemical and Biophysical Research Communications 293:953-957, 2002).

Choulika et al. teach (1) chimeric nucleases where the DNA binding domains are zinc finger binding domains, or meganuclease recognition sites, and the DNA cleavage domains are domains from restriction endonucleases, and (2) a chimeric nuclease comprising the DNA binding domain of a I-Sce I nuclease and the cleavage domain of a FokI nuclease ([paragraph [0042]). Choulika et al. teach that their invention relates to a method of repairing a specific sequence of interest in chromosomal DNA of a cell and teach a targeting DNA that comprises (1) DNA homologous to the region surrounding the site to be targeted in chromosomal DNA, and (2) DNA which repairs the specific sequence of interest upon recombination between the targeting DNA and the chromosomal DNA (paragraph [0026]). It is noted that the term "targeting DNA" of Choulika et al. is equivalent to the term "repair substrate" recited in the claims as both comprise (1) a DNA homologous to the region surrounding the site to be targeted in chromosomal DNA (i.e., nucleic acid sequence that is substantially identical to a region flanking a target sequence in chromosomal DNA), and (2) a DNA which repairs the specific sequence of interest upon recombination (i.e., nucleic acid sequence that replaces the target sequence upon recombination). Choulika et al. also teach a vector comprising a nucleic acid encoding the chimeric nuclease and the targeting DNA (paragraph [0049]; paragraph [0044]). Choulika et al. teach that the vector is a viral vector (paragraph [0045]), the vector has an inducible promoter (paragraph [0046]), and an isolated mammalian cell comprising said vector (paragraph [0052]). Choulika et al. do not disclose the use of a nuclear localization signal or a vector comprising a DNA encoding a second chimeric nuclease.

Bibikova et al. discloses that when cleavage of a chromosomal target is desired, it is very unlikely that exact inverted repeats of a 9-bp sequence will be located in favorable positions, thus there is a need to devise nucleases with two different sets of zinc fingers designed to bind two different 9-mers (page 294, left column, Cleavage of paired nonidentical recognition sites). Bibikova et al. teach the use of two chimeric nucleases with different binding specificities (Zinc finger DNA binding domains) that dimerize and together collaborate to stimulate recombination when their individual sites were appropriately placed

(Abstract; Materials and Methods, Enzymes). The chimeric nucleases are hybrids between the cleavage domain of FokI and a DNA binding domain made up of three Cys_2-His_2 zinc fingers (page 289, last paragraph). Bibikova et al. further disclose that because the recognition of DNA by zinc fingers is modular (each finger contacts primarily three consecutive base pairs in the target), they have been modified to create combinations with novel specificities (page 289, last paragraph). According to Bibikova et al., randomization of the codons for the recognition residues allows selection of new fingers that have high affinity for arbitrarily chosen DNA sequences (page 289, last sentence). Bibikova et al. tech that engineered zinc fingers have been shown to act on their designed targets in living cells and that nucleases based on zinc fingers should be targetable to specific but arbitrary recognitions sites (page 290, first 6 lines). Bibikova et al. teach that for specific cleavage to occur, dimerization of the cleavage domains from both chimeric nucleases is necessary, thus the two recognition sites for the zinc fingers must occur in close proximity (Abstract; page 295, left column, first full paragraph). Bibikova et al. teach that the chimeric endonucleases based on zinc fingers are capable of finding their recognition sites in oocytes, directing specific cleavage, and stimulating local homologous recombination (page 290, right column, first full paragraph). Bibikova et al. also teach the direct injection of these chimeric nucleases and DNA substrates for recombination directly into the nucleus of oocytes (Materials and Methods, Oocyte injections). Bibikova et al. teach that because the recognition specificity of zinc fingers can be altered experimentally, their approach holds great promise for inducing targeted recombination in a variety of organisms (Abstract, last sentence). Bibikova et al. do not teach a single vector comprising DNA encoding the two chimeric nucleases and the DNA substrate.

Takeuchi et al. teach a vector comprising DNA encoding Flp recombinase linked to a nuclear localization signal to increase the efficiency of the recombination process (page 954, right column, Results). Takeuchi et al. do not teach a vector comprising DNA encoding one or two chimeric nucleases and a DNA substrate.

Claim 21 is directed in part to a viral vector comprising a DNA encoding two chimeric nucleases which form a dimmer and cleave an endogenous mammalian gene, wherein each chimeric nuclease comprises a zinc finger DNA binding domain that binds to a target region in the endogenous mammalian gene, a nuclear localization signal, and a FokI cleavage domain, wherein said viral vector further comprises a DNA repair substrate, wherein said substrate comprises a nucleic acid sequence which is substantially identical to a region flanking a target in the endogenous mammalian gene, and wherein said substrate also comprises a nucleic acid sequence which integrates into the cleavage site upon cleavage by the chimeric nucleases and recombination between the repair substrate and the target in the endogenous mammalian gene. Claim 28 is directed to an isolated mammalian cell comprising two chimeric nucleases which form a dimer and cleave an endogenous mammalian gene, wherein each chimeric nuclease comprises a zinc finger DNA binding domain that binds to a target region in the endogenous mammalian gene, a nuclear localization signal, and a FokI cleavage domain, wherein said mammalian cell further comprises a DNA repair substrate, wherein said substrate comprises a nucleic acid sequence which is substantially identical to a region flanking a target in the endogenous mammalian gene, and wherein said substrate also comprises a nucleic acid sequence which integrates into the cleavage site upon cleavage by the chimeric nucleases and recombination between the repair substrate and the target in the endogenous mammalian gene. Please note that the term "sequence which is integrated into the cleavage site upon cleavage by the chimeric nucleases and recombination between the repair substrate and the target sequence" as recited in claims 21 and 28 is the same as a sequence which replaces the target sequence upon recombination as that is the actual function of the repair substrate upon cleavage of the target site, i.e., the replacement of what is at the cleavage target site with the repair substrate.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the viral vector of Choulika et al. such that it would encode two chimeric nucleases that dimerize (as taught by Bibikova et al.) wherein the chimeric nucleases comprise a zinc finger DNA

binding domain, a FokI cleavage domain, and a nuclear localization signal (taught by Takeuchi). Also, it would have been obvious to one of ordinary skill in the art at the time the invention was made to transform an isolated mammalian cell with the viral vector described above such that the chimeric nucleases could be expressed and also to provide the repair substrate.

A person of ordinary skill in the art is motivated to (1) add a DNA encoding a nuclear localization signal to the vector of Choulika et al. in view of the teachings of Takeuchi et al., who disclose that adding a nuclear localization signal enhances recombination, (2) use chimeric nucleases which comprise zinc finger DNA binding domains and the FokI cleavage domain in view of the teachings of Choulika et al., who disclose chimeric nucleases comprising zinc finger DNA binding domains as well as chimeric nucleases having the FokI cleavage domain, and also in view of the teachings of Bibikova et al., who teach chimeric nucleases comprising both zinc finger DNA binding domains and the FokI cleavage domain, and (3) use two chimeric nucleases both having zinc finger DNA binding domains and the FokI cleavage domain in view of the teachings of Bibikova et al. who disclose that using two chimeric nucleases having two sets of three zinc binding fingers, allows for the possibility of directing cleavage to many more targets, and (4) use a single vector to deliver the nucleic acids encoding the chimeric nucleases and the repair substrate to a host cell for the benefit of delivering all the required components in a single vehicle thus reducing the number of transformation/transduction events to one. Also, a person of ordinary skill in the art is motivated to introduce the vector of Choulika et al., Bibikova et al. and Takeuchi et al. in an isolated mammalian cell for the benefit of targeted recombination, in view of the teachings of Choulika et al. (paragraph [0004]) and Bibikova et al. (page 289, left column, second paragraph), who teach that making an intentional double strand break in target DNA increases homologous recombination events.

One of ordinary skill in the art has a reasonable expectation of success at making the vectors and transforming isolated mammalian cells with said vectors since (1) Choulika et al. and Takeuchi et al.

teach the construction of vectors comprising DNA encoding chimeric nucleases and nuclear localization signals, respectively, (2) Choulika et al. already teach a vector that comprises both the nucleic acid encoding the chimeric nuclease and the repair substrate, (3) Bibikova et al. teach vectors encoding each of the chimeric nucleases comprising Zinc finger DNA binding domains, and (4) the molecular biology techniques required to make vectors and transform isolated mammalian cells with said vectors are well known and widely used in the art. Therefore, the invention as a whole would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made.

6. Applicant submits that the amendments to claims 21 and 28 obviate the instant rejection because the Board upheld the previous rejection partly on the grounds that claim 28 is not a method claim and claim 28 does not positively recite an isolated mammalian cell wherein a repair substrate has been integrated into its genome in a targeted (site-specific) location. Applicant argues that the claims require two zinc finger nucleases which dimerize that specifically target sites in an endogenous mammalian gene, and that the repair substrate is integrated in a targeted matter at the cleavage site. Applicant argues that the teachings of Porteus indicate that zinc finger nucleases that form dimers to cleave endogenous mammalian targets and integration into endogenous mammalian genes was not predictable at the time of the invention.

7. Applicant's arguments have been fully considered but not deemed persuasive to overcome the instant rejection. For the record, it is not believed that the Board upheld the previous rejection partly on the grounds that claim 28 is not a method claim and does not positively recite that a repair substrate has been integrated into its genome in a targeted location. On pages 14-15 of the decision, the Board stated the following:

> According to Appellants, "Porteus clearly teaches that it was not until 2005 that ZFN-mediated targeted integration in mammalian cells was actually shown" and, therefore, "Choulika and Bibikova cannot teach that the claimed methods were predictable, when in 2005, the skilled artisan was stating that the first proof of targeted integration in mammalian cells was available" (Reply Br. 11). However, claim 28 is not a method claim and claim 28 does not positively recite an isolated mammalian cell wherein a repair substrate has been integrated into its genome in a targeted (site-specific) location. Similarly, the teachings of Porteus regarding homing endonucleases is not on point (FF 15-16) because the claims on appeal require chimeric zinc finger nucleases. Therefore, on balance, Porteus is insufficient to overcome a reasonable expectation of success that a chimeric nuclease would cleave chromosomal DNA in an isolated mammalian cell provided by the teachings of Choulika and Bibikova to one of ordinary skill in the art.

This statement is simply indicating what claim 28 is not reciting. It says nothing about Porteus et al. (Nature Biotechnology 23(8):967-973, 2005) being evidence that it would be unpredictable for zinc finger nucleases to form dimers and cleave endogenous mammalian targets in an "isolated mammalian cell", or that integration of a repair substrate into the cleavage site of a mammalian gene in an "isolated" mammalian cell was not predictable at the time of the invention. It has been extensively argued by the Examiner that the use of two zinc finger nucleases that dimerize is taught by Bibikova et al. See discussion above. Furthermore, integration of a repair substrate at the cleavage site in an isolated cell has been shown to occur as evidenced by Choulika et al. (NIH 3T3 from mouse) and Bibikova et al. (Xenopus oocytes). The only difference between the prior art cited and claim 28 as amended is the requirement that the target be an endogenous mammalian gene. The requirement regarding the integration of the repair substrate into the cleavage site has been interpreted as being equivalent to the repair substrate being able to replace the target, which is the definition of a repair substrate, as extensively discussed above. As extensively argued by the Examiner in the past and most recently in the Examiner's answer of 7/7/2009,

it is abundantly clear from the teachings of Choulika et al. that the intended target of Choulika et al. is an endogenous chromosomal DNA or gene. The working example of Choulika et al. which was carried out in an isolated mammalian cell clearly demonstrated the principle that one could target the chromosome of a host cell for repair, thus at a minimum suggesting the endogenous limitation. Similarly, while Bibikova et al. do not teach a working example of an endogenous mammalian chromosomal target, Bibikova et al. laid out the experimental procedure to follow for chromosomal gene targeting (page 296 of Bibikova et al.). Thus, at a minimum, Bibikova et al. suggest cleavage of an endogenous target. It is noted that while the Examiner has deemed the invention of claims 21 and 28 as enabled by the teachings of the specification and the prior art, it is noted that if the argument is made that it was not until 2005 that a vector and isolated mammalian cells as currently claimed were possible, and Porteus is one of the inventors of the instant application and one of the authors of the cited reference, one would have to question as to whether the current claims are enabled.

Conclusion

8. No claim is in condition for allowance.

9. Certain papers related to this application may be submitted to Art Unit 1652 by facsimile transmission. The FAX number is (571) 273-8300. The faxing of such papers must conform with the notices published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 CFR 1.6(d)). NOTE: If Applicant submits a paper by FAX, the original copy should be retained by Applicant or Applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED, so as to avoid the processing of duplicate papers in the Office.

10. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PMR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through

Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Delia M. Ramirez, Ph.D., whose telephone number is (571) 272-0938. The examiner can normally be reached on Monday-Friday from 8:30 AM to 5:00 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert B. Mondesi, can be reached at (571) 272-0956. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571) 272-1600.

/Delia M. Ramirez/

Primary Patent Examiner
Art Unit 1652

DR
May 2, 2011